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Food and Health Applications of Exopolysaccharides produced by Lactic acid Bacteria

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Abstract

Majority of the polysaccharides used in foods are of plant, animal and algae origin. The exopolysaccharides (EPS) produced by food grade lactic acid bacteria (LAB) have gained much importance as biothickeners and texturizers in recent time. Several animal studies and *in vitro* tests are also suggestive of beneficial health effects associated with the regular consumption of EPS producing LAB. Such biopolymers are not synthesized in abundant amounts, which are a major factor impacting upon production cost and recovery time, considered as major constraints to full commercialization of these technologically important biopolymers. In current article, we reviewed prospective food and health applications of bacterial EPS.

Keywords: Exopolysaccharides; Prebiotic; *Weissella*; Antitumour; Fermented foods; Biosorption

Introduction

Polysaccharides may function in foods as viscosifying agents, stabilizers, emulsifiers, gelling agents, or water-binding agents. The global market for hydrocolloids, which includes many polysaccharides, is still dominated by plant and algae derived polysaccharides such as starch, galactomannan, pectin, carrageenan and alginate [1]. This market valued at >4 million US$ in 2008, with xanthan gum being the only significant bacterial exopolysaccharide (EPS), which accounted for 6% of the total market value [2]. However, most of them are chemically or enzymatically modified in order to improve their rheological properties, e.g. cellulose, starch, pectin, alginate and carrageenan and therefore, their use is strongly restricted for food applications. An alternative source of biopolymers is microbial EPS. The EPS of microbial origin have unique rheological properties because of their capability of forming very viscous solutions at low concentrations and their pseudoplastic nature [3]. Commercially available microbial EPS are xanthan and gellan which are produced by *Xanthomonas campestris* and *Pseudomonas elodea*, respectively. Products containing these EPS are required to be labelled since these bacteria are plant pathogens.

Inherently, Lactic acid bacteria (LAB) are associated with many fermented foods; particularly milk based products such as curd, yoghurt, sour cream, cheese and buttermilk where they contribute to develop taste, flavour and shelf life of fermented foods [4]. LAB possess generally regarded as safe (GRAS) status which allows them to be incorporated in food without labelling. Some strains of LAB have been reported to produce EPS and gained increasing attention over the last few years because of their contribution to the rheology and texture of fermented milk and food products [5]. Most of the LAB producing EPS belongs to the genera *Streptococcus, Lactobacillus, Lactococcus, Leuconostoc*, and *Pediococcus*. Production of EPS is also reported from some non starter LAB like *Bifidobacteria*. LAB are able to produce mainly two types of polysaccharides according to their location in the cell, intracellular polysaccharides and extracellular polysaccharides [6]. Some bacteria produce only capsular EPS, some produce only slime (ropy) form, whereas, in some cases, bacteria can produce both forms of EPS [7,8].

EPS impart highly desirable rheological changes in the food matrix such as increased viscosity, improved texture and reduced syneresis [9]. EPS-producing LAB have a greater ability to withstand technological stresses [10] and survive the passage through the gastrointestinal tract compared to their non-producing bacteria. Further, EPS may induce positive physiological responses including lower cholesterol levels [11,12], reduced formation of pathogenic biofilms [13], modulation of adhesion to epithelial cells [14] and increased levels of *bifidobacteria* showing a prebiotic potential [15,16]. Hence, the choice of EPS-producing starter cultures seems to give several advantages over non-producing ones.

EPS in Food Applications

Various microbial and plant derived biopolymers with their applications in different fields are summarized in Table 1. Dextran is the first industrial polysaccharide produced by LAB like *Leu. mesenteroides*. It was discovered in 1880 in sugar cane or beet syrups where dextran was found to be responsible for the thickening and gelation of the syrups [17]. Due to their structural differences, some dextrans are water soluble and others are insoluble. Dextran can be used in confectionary to improve moisture retention, viscosity and inhibit sugar crystallization. In gum and jelly candies, it acts as a gelling agent. In ice cream it acts as a crystallization inhibitor, and in pudding mixes it provides the desirable body-texture and mouth feel [18]. In addition, dextran has also been used as blood plasma extenders [19] and as the basic component of many chromatographic stationary phases [20]. Dextran from *Leu. mesenteroides* protects the producer strain during starvation and helps in survival at alkaline and acidic conditions [21].

Xanthan gum, produced by the plant-pathogen, *Xanthomonas campestris* is the second microbial EPS which was approved for use in

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Biopolymer	Monomer units	Producer Organism	Applications
Dextran	Glucose	*Leuconostoc mesenteroides*	In confectionary products to improve moisture retention, viscosity and inhibit sugar crystallization, In veterinary and human medicine as blood plasma extender or blood flow improving agent and as cholesterol lowering agent, in separation technology and in aqueous two phase systems, as micro-carrier in tissue/cell culture
Xanthan	Glucose, mannose, glucuronic acid	*Xanthomonas*	As viscosifier, stabilizer, emulsifier and suspending agent in food industries, in food as thickening and stabilizing agent- often used in combination with guar gum, in secondary and tertiary crude-oil recovery, in paints, pesticide and detergent formulations, pharmaceuticals, cosmetics, printing inks (to control viscosity, settling and gelation),
Levan	Fructose	*Bacillus, Streptococcus, Pseudomonas, Zymomonas*	For production of sweet confectionary, ice cream as viscosifer and stabilizer
Alginate	Guluronic acid, mannuronic acid	*Pseudomonas aeruginosa and Azotobacter vinelandii*	As an immobilization matrix for viable cells and enzymes, coating of roots of seedlings and plants to prevent desiccation, micro-encapsulation matrix for fertilizers, pesticides and nutrients, hypo-allergic wound-healing tissue
Emulsan	Sugar and fatty acid	*Acinetobacter calcoaceticus*	crude-oil recovery and other applications are similar as for alginate
Gellan	Glucose, rhamnose, glucuronic acid	*Sphingomonas paucimobilis*	As stabilizer and suspending agent for foods. As gelling agent for solidifying culture media, especially for studying marine microorganisms
Cellulose	Glucose	*Acetobacter spp.*	In human medicine as temporary artificial skin to heal burns or surgical wounds, in nutrition as natural non-digestible fibers (which can be impregnated with amino acids, vitamins and minerals), in separation technology, as acoustic membranes in audio-visual equipment
Curdlan	Glucose	*Rhizobium meliloti and Agrobacterium Radiobacter*	As a gelling agent, immobilization matrix, curdlan along with zidovudine (AZT), displays promising high antiretroviral activity (anti AIDS-drug)
Hyaluronic acid	glucuronic acid, N-acetyl glucosamine	*Streptococcus equii and Streptococcus zooepidemicus*	As replacer of eye fluid in ophthalmic surgery, in artificial tear-liquid, synovial fluid replica, in wound healing, cosmetic industry (lotions, moisturizing agent)
Succinoglycan	Glucose, galactose	*Alcaligenes faecalis var. myxogenes*	As a gelling agent and other functions are same as for curdlan
Acetan	Cellobiose, glucose	*Acetobacter xylinum*	As viscosifier and gelling agent for food. For production of sweet confectionary and vinegar.
Glucuronan	Glucuronic acid polymer	*Sinorhizobium meliloti M5N1CS, Gluconacetobacter hansenii*	Food and cosmetics products
Colanic acid	Fucose, glucose, glucoronate, and galactose	*E. coli, Shigella spp., Salmonella spp., Enterobacter spp.*	Cosmetics and personal care products

Table 1: Microbial biopolymers and their applications (Kumar et al. [36] and van Kranenburg et al. [37]).

foods in 1969. Xanthan has been described as "benchmark" product with respect to its importance in both food and non-food applications [22], which include dairy products, drinks, confectionary, dressing, bakery products, syrups and pet foods, as well as the oil, pharmaceutical, cosmetic, paper, paint and textile industries. The production of xanthan is relatively inexpensive because of the high conversion of substrate (glucose) to polymer (60-70%) [22]. According to Becker et al. [3], this biopolymer exhibits a high viscosity at low concentrations in solution and strong pseudoplasticity, and stable over a wide range of pH, temperature and ionic strength.

Microbial polysaccharides have rheological properties that match the industrial demands and can be produced in large amounts and high purity. Since 1940, dextran and levan have found numerous pharmaceutical and food applications. Fructose-oligosaccharides (FOS) have interesting properties for food applications as they have a low sweetness compared to sucrose, are essentially calorie-free, and noncariogenic [23]. Food applications of inulin and FOS are based mainly on their prebiotic properties. Remarkably, fructose based polymers have ability to be fermented by gut microflora which, leads to improvement of the intestinal flora and increases mineral absorption [24,25]. The levan from *L. sanfranciscensis* LTH 2590 also exhibits prebiotic effects as demonstrated *in vitro* by different experimental approaches [15,26]. Fructans play a role in the cellular stress tolerance of plants through stabilization of membranes [27]. The property of FOS and EPS to protect bacteria against various stresses enable their application to improve the survival and activity of LAB during the preparation and storage of fermented milk products.

EPS produced by LAB, are widely used to improve the body and texture of yoghurt and other fermented milk products like dahi [8,28]. Dahi is a popular fermented milk product of India having 3.5-8% fat, similar to yoghurt, consumed in almost every household [29]. Health awareness among consumers generated more demands for low-caloric, especially low-fat or fat-free dairy products in market. However, since milk fat contributes to the flavour, body and texture development of the dairy products, removal leads to textural and functional defects in low fat fermented milk products. In case of low fat yoghurt and dahi, a lack of flavour, weak body and poor texture are the major problems [30]. In this perspective, EPS producing LAB as 'biothickeners' offer natural, more acceptable and preferred approach to many additives. These cultures meet the consumer requirement for products with low levels of chemical additives [31,32], reduce the amount of total solids required without affecting the textural attributes [31,33] and improves sensory properties [34]. Low fat dahi made using different EPS producing cultures of L. *Lactis* subsp. *Lactis* PM23, *S. thermophilus* ST and *L. Lactis* NCDC 191 found to be more acceptable in terms of body, texture and flavour as compared to dahi made with EPS negative culture NCDC 167 [35].

The microstructural studies showed that dahi made with EPS-producing strains had more open structure and pores with discontinuous casein matrix than the controlled dahi, which had relatively compact linear structure [38]. To overcome the defects of low fat cheeses, manufacturers have used texture promoting or ropy cultures for many years particularly where addition of stabilizer is prohibited. Such ropy strains may impart higher flavour intensity in the fermented milk due to the carbohydrate masking the flavour, mouth feel and other attributes may also be affected. The apparent viscosity of skim milk gel made by two ropy cultures, namely B-6 and KT-24 (later identified as *Lc. Lactis* subsp. *Lactis* strains) was increased as compared to that made by non-ropy cultures [35,39]. Recently, Florencia [40] studied rheology of spreadable goat cheese made with autochthonous lactic cultures differing in their ability to produce EPS and concluded that cheese made with EPS producing strain showed smaller elastic or storage module (G'), viscous or loss module (G"), and complex viscosity (η*) values over the range of frequencies studied and smaller critic stress values than the cheese without EPS producing strain.

EPS-producing *L. rhamnosus* JAAS8 in combination with non-EPS-producing yogurt strains increased both the water-holding capacity (WHC) and viscosity of the fermented products when compared with the control made with non-EPS-producing yogurt starters [41]. Partial or total replacement of non-EPS-producing *L. delbrueckii* subsp. *bulgaricus* with EPS-producing *L. rhamnosus* JAAS8 resulted in significant increases of about 16% and 21%, respectively, in apparent viscosity and an increase of about 2% in the WHC of the fermented products. To reduce the amount of added milk solids, to improve yoghurt viscosity, to enhance texture and mouth feel and to avoid syneresis during fermentation or upon storage of the fermented milk products, EPS producing functional starters are interesting. Yogurt made with EPS producing cultures has better water binding capacity, which decreases the product's susceptibility to syneresis [42-44]. However, no simple correlation has been established between viscosity and quantity of EPS produced. It is opined that it may be more beneficial to use a combination of ropy and non-ropy starter cultures than using only ropy strains [45].

Besides dahi, yoghurt and cheeses, the other fermented milk products in which EPS cultures have been shown to affect product rheology are sour cream, kefir, and European cultured dairy products. Use of slime producing *S. thermophilus* strains greatly improved rheological properties of cream turo and number of other Hungarian cultured milk and cultured cream products [46]. Kefir is traditional self-carbonated, slightly alcoholic fermented milk from Eastern Europe [4,47]. Kefir is prepared using kefir grains which consist of homofermentative and heterofermentative LAB, yeasts and acetic acid bacteria. These cells are embedded in kefiran, a slimy polysaccharide, which also found to affect texture of kefir [48]. Furthermore, some associated LAB strains also produces EPS within the matrix acting as natural viscosifying agent.

Dextran from *Leu. mesenteroides* finds commercial application in baking improvers. A study performed by Brandt et al. [49] provided evidence that EPS effectively improve dough rheological parameters and bread quality. Remarkably, EPS produced *in situ* was more effective when compared to externally added levan and addition of 1% (flour base) sucrose to wheat doughs sufficed to induce polymer formation by *Lactobacilli* to effective concentrations. The *in situ* formation of EPS from sucrose resulted in further metabolites such as manniitol, glucose, and acetate that may contribute to the improved bread quality [50]. The EPS producing strains of *Weissella* in sourdoughs improved the textural properties and quality of bread [51,52]. Polymers produced from *Lactobacilli* thus may be expected to beneficially affect one or more of the following technological properties of dough and bread: (i) water absorption of the dough, (ii) dough rheology and machinability, (iii) dough stability during frozen storage, (iv) loaf volume and (v) bread staling.

During prolonged fermentation and storage, idli batters start collapsing and whey is separating that leaves idli with a hard, unwanted texture. Addition of different hydrocolloids in the batter may help to improve the texture of the final product [53]. Xanthan at a concentration of 0.1% improved the textural properties resulting in sensory analysis scores that are higher compared to idli without additives [54]. Nisha et al. [53] added different hydrocolloids to idli batter where all gave an increased viscosity. The decrease in batter volume during storage was less compared to the control and less whey separation was reported. However, only agar and guar gum gave idli that were acceptable from a sensory point of view with the right mouth feel and texture. Starter cultures producing EPS may be used to provide natural stabilizing agents *in situ*. EPS may work as natural thickening agents, giving the product a higher viscosity and reducing syneresis [55] thus giving an improved product without the use of additives.

Physiological Functions of EPS

Apart from their industrial applications, EPS have been found to be associated with many additional functions in microbial cell. However, the physiological role of EPS in bacteria has not been clearly established, and is probably diverse and complex. The precise role of the EPS, apparent in different ecological niches, is dependent on the natural environment of the microorganism. In general, microbial EPS are thought to play a role in the protection of the microbial cell in their natural environment against adverse condition such as desiccation, osmotic stress, antibiotics or toxic compounds (e.g. toxic metal ions, sulphur dioxide, and ethanol), predation by protozoans, phagocytosis and phage attack. The ability of a microorganism to surround itself with a highly hydrated exopolysaccharide layer may provide it with protection against desiccation and predation by protozoans [36]. Also, the presence of a gelled polysaccharide layer around the cell may have paramount effects on the diffusion properties, both into and out of the cell [56]. Some researchers have claimed that cell-associated EPS reduces sensitivity of the bacteria against bacteriophages and lysozyme, most likely by masking the targets for the phages and the enzyme [57,58]. Bacterial strains with relatively high EPS producing capacity were insensitive against phages and nisin suggesting their beneficial physiological function in natural environment [57].

Stack et al. [10] analyzed the ability of β-glucan-producing *L. paracasei* NFBC 338 to survive both technological and gastrointestinal stresses. Heat stress assays revealed that production of the polysaccharide was associated with significantly increased protection during heat stress (60-fold), acid stress (20-fold), and simulated gastric juice stress (15-fold). While bile stress assays revealed a more modest but significant 5.5 fold increase in survival for the β-glucan producing strain compared to that of the control strain. These results propose that production of a β-glucan EPS by strains destined for use as probiotics may afford them greater performance /protection during cultivation, processing, and ingestion. Similar to this, in one of the *in vitro* experiments, Lebeer et al. [59] suggested that EPS of *L. rhamnosus* GG form a protective shield against innate immune factors in the intestine. The mutant strain (with no EPS production trait) was more sensitive towards host innate defence molecules, such as the LL-37 antimicrobial peptide and complement factors. This suggests that EPS forms a protective shield for LGG against these molecules in the GI tract.

The production of EPS in the form of capsules is eminent in pathogenic bacteria, wherein the pathogenicity of an organism depends on the rate of synthesis and the amount of EPS synthesized. Capsules attached with pathogen enable evasion of phagocytosis. A noteworthy fact is that all capsular polysaccharides do not activate the immune system, which is due to the fact that their chemical structures may mimic the host cell surface components [36]. The lectins, polysaccharide binding proteins secreted by the plant, (e.g. Trifolin A) play a crucial role in the establishment of the symbiotic association between *Rhizobium* spp. and leguminous plants [60]. EPS may help bacterial cells for adhesion to solid surfaces and biofilm formation, and also in cellular recognition. It is not likely that EPS serves as a food reserve for the bacteria producing them, because most slime-forming bacteria are not capable of metabolizing the EPS they produce [61]. Apart from protection, few studies on EPS suggested that EPS produced by specific LAB could exert health beneficial effects through immunomodulation or by other means [36,62,63], some of these observations are discussed below.

Prebiotic effect

The possibility of acting as prebiotic substrates has been demonstrated successfully by Korakli et al. [26] for a fructan-type EPS produced by one strain of *L. sanfranciscensis*. There was evidence of a bifidogenic effect for the levan-type EPS produced by another strain of the same species [15]. Another experimental approach carried out by Salazar et al. [64] showed that EPS synthesized by intestinal *Bifidobacteria* act as fermentable substrates for microorganisms in the human gut environment, modifying interactions among intestinal populations.

Antigastritis, antiulcer and cholesterol lowering effects

Purified EPS from *S. thermophilus* CRL 1190 was found to be effective for preventing chronic gastritis [63]. The authors justified that the EPS-protein interaction might be responsible for the observed gastroprotective effect; such interactions may be affected by industrial manufacturing conditions. Similarly, Nagaoka et al. [65] reported certain antiulcer effects of EPS produced by *bifidobacteria*, lactobacilli, and streptococci strains. However, the protection of the gastric epithelium by EPS-producing LAB has not yet been described in detail.

The milk fermented with an EPS-producing strain *Lc. Lactis* ssp. *cremoris* SBT0495 had cholesterol lowering activity, however, the mechanism is unknown [66]. In another research, the adsorption of total cholesterol by polysaccharides was measured *in vitro* by enzymatic reactions, including the polysaccharide precipitation procedure [67]. They mentioned that with 0.1% (wt/vol) polysaccharide dissolved in distilled water, the adsorption capacities of alginate, pectins, gellan gum, xanthan gum, and zooglan were 2.9, 2.88, 2.5, 2.9, and 2.4 mg/dL, respectively. However, 0.2% of zooglan was able to completely adsorb the cholesterol (3 mg/dL), whereas dextran could not. Tok and Aslim [68] also stated that out of total 5 strains of *L. delbrueckii* subsp. *bulgaricus*, isolated from home-made yoghurt, three strains that were producing high amounts of EPS were able to remove more cholesterol from the medium compared to low EPS producer strains. They reported that immobilized cells were much effective in cholesterol adsorption than free cells.

Anti-mutagenic properties

EPS-bound cells of strain *L. plantarum* showed binding ability to mutagens such as heterocyclic amines, and the mutagens were inactivated by binding to EPS [69]. There are very scarce studies on experimental animals to study either physiological or immunological effects by feeding or dosing EPS. In one of the animal studies, changes in blood pressure and serum components were examined in SHRSP/Hos rats using doses of 100 and 300 mg of kefiran/kg of rat [12]. After 30 days, suppression in the increase in blood pressure was reported.

Adhesion and colonization

Studies on bacterial adhesion showed that capsular polysaccharide might promote the adherence of bacteria to biological surfaces, thereby facilitating the colonization of various ecological niches. The EPS were found to be present in adherent biofilms [36,70]; the EPS might function as initial adhesion, and permanent adhesion compounds [71]. Furthermore, EPS may function as adhesive agents and facilitate interactions between plants and bacteria, i.e., levan production by the root of the sugar cane invading *G. diazotrophicus* [72]. Homopolysaccharides (glucans and fructans) formed by oral streptoccocci have a major influence on the formation of dental plaque. They are involved in adherence of bacteria to each other and to the tooth surface, modulating diffusion of substances through plaque and serving as extracellular energy reserves [73]. Furthermore, fructans aid in the adhesion of bacterial cells to plant cell surfaces [72]. Although the involvement of these biopolymers in bacterial adhesion to the intestinal epithelium has not yet been validated through *in vivo* studies, some *in vitro* studies suggests that EPS could lead to increase the efficiency of adhesion to the epithelial layer of the GI tract by the LAB [74]. It has been known that the EPS of *Streptococcus mutans* play significant role for the adhesion of this bacterium to the tooth surface and hence in the formation of dental plaque [75], facilitating bacterial colonization and protection against hostile habitats. Several studies indicate that EPS produced by LAB appear to be associated in cellular recognition, adhesion and the formation of biofilms [55,75]. Adhesion of probiotic *Lactobacilli* cultures to HT29 cells was reported by Vishwanth et al. [76] by flow cytometry. These cultures are known to produce EPS.

Antitumour properties

Oda et al. [77] reported an antitumor EPS produced by *L. helveticus* ssp. *jugurti*. The antitumor activity of the EPS was tested against ascites Sarcoma-180 by injecting the EPS preparation intraperitoneally. Mice given a 20 mg kg^{-1} dose for nine successive days had an increased life span value of 144%, and a value of greater than 233% corresponding to a 40 or 80 mg kg^{-1} dose. The authors concluded that the antitumor activity of the EPS might be based on its host-mediated actions. In order to understand the antitumor activity, the effect of the EPS or the EPS-producing cells on the immune system has been investigated. Forsen et al. [78] showed that cell surface materials, possibly lipoteichoic acids, of *Lc. Lactis* ssp. *cremoris* T5 produced T-cell mitogenic activity in human lymphocytes. Few studies also reveal immunomodulating, and antitumor activities of EPS [62,79,80]. The slime produced by *B. adolescentis* had immunomodifying effects on mouse splennocytes [81]. Kitazawa et al. [82] showed that the slime-forming *Lc. Lactis* ssp. *cremoris* KVS20 had antitumor activity, and the slime contained strong B-cell dependent mitogenic substances.

Miscellaneous applications

Entertoxigenic *E. coli* (ETEC) is a big threat to swine industry. A recent study performed by Wang et al. [83] showed that EPS produced by strains of *L. reuteri* inhibited ETEC-induced heamagglutination of porcine erythrocytes suggesting potential application of EPS associated with probiotic strain to inhibit pathogenicity of ETEC. However, no effect was observed for dextran produced from *Weissella cibaria* and commercially available oligo- and polysaccharides in the same study. A low molecular weight heparin-like EPS exhibiting anticoagulant

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property has been reported from *Alteromonas infernus*, obtained from deep-sea hydrothermal vents [84]. Clavan, an L-frucose containing polysaccharide has a potential application in preventing tumor cell colonization of the lung, in controlling the formation of white blood cells, in the treatment of the rheumatoid arthritis, in the synthesis of antigens for antibody production and in cosmeceuticals as skin moisturizing agent [85]. The EPS of *L. paracasei* subsp. *paracasei* NTU 101 and *L. plantarum* NTU 102 demonstrated potential antioxidant properties including *in vitro* 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity, chelation of ferrous ions, inhibition of linoleic acid peroxidation, and reducing power [86]. EPS also found to stimulate cell proliferation and mild immune modulator of macrophages by inducing cytokine production (including IL-6, TNF-α, and IL-1β) in a dose-dependent manner (5-500 μg mL^{-1}). Furthermore, studies by Martin et al. [87] show that some bacterial EPS alone or as conjugates could act as a very potent somnogen, thus sleep induction with a natural product with no side effects will eliminate dependence on xenobiotics for sleep induction.

Future Prospects for Bacterial EPS

The industrial application of bacterial EPS is limited due to higher production cost and recovery processes. Though streptococci and related genera produces EPS in very less amount, most of the *Lactobacilli* are reported to produce 1-10 g of EPS per litre growth media [88,89], while *Weissella* species produce EPS in amount from a few grams up to 18 g/l under optimized conditions [90]. Such higher EPS producing strains can be explored to resolve the cost related problem. On the other hand, Pediococci and related genera have been found to produce EPS in few milligrams per litre in their favourable media as reported by Patel et al. [88].

Although bacterial EPS applications spans through areas such as the industry (textile, dairy, cosmetics, etc.), health (medicine and pharmaceuticals) and environment (remediation, flocculation, etc.); its application in the flocculation process will be a significant milestone to health promotion and eco-friendly usage especially in municipal and wastewater treatment processes. The flocculations of suspended particles in water treatment plants have applied the use of inorganic salts of aluminium such as aluminium sulphate and poly-aluminium chloride and organic synthetic polymers of polyacrylamide derivatives and polyethyleneimine. These flocculants have been shown to possess adverse health effects such as neurotoxicity, carcinogenicity and Alzheimer's disease [91]. Microbial EPS could serve as safe alternative flocculating agents. Several researchers have reported high flocculation efficiency mediated by the biopolymers produced by non lactic bacteria like *VirgiBacillus* spp, *Bacillus* spp. and *Artrobacter* spp. [92,93]. These findings imply that bacterial EPS effectively mediate flocculation and thus may be applied in large scale industrial processes, with particular reference to water and wastewater treatment. Studies should be designed to check the feasibility of EPS produced by LAB for such eco-friendly approaches.

EPS may also contribute to the provision of reduced oxygen tension and participate in the uptake of metal ions [60,94]. It was found that EPS obtained from *Pseudoalteromonas* spp. SM9913 (psychrotolerant bacterium) was found to enhance the stability of the cold-adapted protease MCP-01 secreted by the same strain through preventing its autolysis. The EPS could bind many metal ions, including Fe^{2+}, Zn^{2+}, Cu^{2+} and Co^{2+} [95]. It also worked as a very good flocculating agent and could conglomerate colloidal and suspended particles.

Conclusion

Bacterial exopolysaccharides shows enormous diversity. In addition to their natural protective role, EPS produced by LAB is found to shield the cell against technological stresses during manufacturing fermented milk products. Recent development is suggestive of potential application of these polymers for human usage; medical, cosmetics, pharmaceutical, dairy products and other forms of industrial and environmental aspects. However, production cost is the limiting factor for application of several prospective exopolysaccharides at industrial level. The search for high EPS producing strains is an ongoing process, while optimizing the fermentation conditions, biotechnological tools involving genetic and metabolic engineering as well as the exploration of cheap fermentation substrates for their production are optional methods for improving the commercial scale production and field application of microbial biopolymers.

References

1. Freitas F, Alves VD, Reis MA (2011) Advances in bacterial exopolysaccharides: from production to Biotechnological applications. Trends Biotechnol 29:388-398.
2. Imeson A (2010) Food stabilisers thickening and gelling agents. Wiley-Blackwell (ed) 10-343.
3. Becker A, Katzen F, Pühler A, Ielpi L (1998) Xanthan gum biosynthesis and application: a biochemical/genetic perspective. Appl Microbiol Biotechnol 50: 145-152.
4. Shah N, Prajapati JB (2013) Effect of carbon dioxide on sensory attributes, physico-chemical parameters and viability of Probiotic *L. helveticus* MTCC 5463 in fermented milk. J Food Sci Technol.
5. Cerning J, Marshall VME (1999) Exopolysaccharides produced by the dairy lactic acid bacteria. Recent Research *Developments in* Microbiology 3: 195-209.
6. Degeest B, Vaningelgem F, De Vuyst L (2001) Microbial physiology, fermentation kinetics, and process engineering of heteropolysaccharide production by lactic acid bacteria. Int Dairy J 11:747–757.
7. Yang Z, Huttunen E, Staaf M, Widmalm G,Tenhu H (1999) Separation, purification and characterisation of extracellular polysaccharides produced by slime-forming *Lactococcus Lactis* ssp. *cremoris* strains. Int Dairy J 9: 631-638.
8. Broadbent JR, McMahon DJ, Welker DL, Oberg CJ, Moineau S (2003) Biochemistry, genetics, and applications of exopolysaccharide production in *Streptococcus thermophilus*: a review. J Dairy Sci 86: 407-423.
9. Badel S, Bernardi T, Michaud P (2011) New perspectives for *Lactobacilli* exopolysaccharides. Biotechnol Adv 29: 54-66.
10. Stack HM, Kearney N, Stanton C, Fitzgerald GF, Ross RP (2010) Association of beta-glucan endogenous production with increased stress tolerance of intestinal Lactobacilli. Appl Environ Microbiol 76: 500-507.
11. Levrat-Verny MA, Behr S, Mustad V, Rémésy C, Demigné C (2000) Low levels of viscous hydrocolloids lower plasma cholesterol in rats primarily by impairing cholesterol absorption. J Nutr 130 243-248.
12. Maeda H, Zhu X, Suzuki S, Suzuki K, Kitamura S (2004) Structural characterization and biological activities of an exopolysaccharide kefiran produced by *LactoBacillus* kefiranofaciens WT-2B(T). J Agri Food Chem 52: 5533-5538.
13. Kim Y, Oh S, Kim SH (2009) Released exopolysaccharide (r-EPS) produced from probiotic bacteria reduce biofilm formation of enterohemorrhagic *Escherichia coli* O157:H7. Biochem Biophys Res Commun 379: 324-329.
14. Ruas-Madiedo P, Gueimonde M, Margolles A, de los Reyes-Gavilán CG, Salminen S (2006) Exopolysaccharides produced by probiotic strains modify the adhesion of probiotics and enteropathogens to human intestinal mucus. J Food Prot 69: 2011-2015.
15. Bello FD, Walter J, Hertel C, Hammes WP (2001) *In vitro* study of prebiotic properties of levan-type exopolysaccharides from *Lactobacilli* and non-digestible carbohydrates using denaturing gradient gel electrophoresis. Syst Appl Microbiol 24: 232-237.
16. Hongpattarakere T, Cherntong N, Wichienchot S, Kolida S, Rastall RA (2012)

In vitro prebiotic evaluation of exopolysaccharides produced by marine isolated lactic acid bacteria. Carbohydrate Polymers 87: 846-852.

17. Crescenzi V (1995) Microbial polysaccharides of applied interest: Ongoing research activities in Europe. Biotechnol Prog 11: 251-259.
18. Whistler R, Daniel JR (1990) Functions of polysaccharides in foods. In: Food Additives eds Branen AL Davidson PM & Salminen S, Marcel Dekker Inc New York 395-423.
19. Kulicke WM, Heinze T (2005) Improvements in Polysaccharides for use as Blood Plasma Expanders. Macromol Symp 231: 47-59.
20. Franz G (1986) Polysaccharides in pharmacy. Adv Polymer Sci 76: 1-30.
21. Kim DS, Thomas S, Fogler HS (2000) Effects of pH and trace minerals on long-term starvation of *Leuconostoc mesenteroides*. Appl Environ Microbiol 66: 976-981.
22. Sutherland IW (1998) Novel and established applications of microbial polysaccharides. Trends Biotechnol 16: 41-46.
23. Yun JW (1996) Fructooligosaccharides—Occurrence, preparation, and application. Enz Microb Technol 19: 107-117.
24. Yamamoto Y, Takahashi Y, Kawano M, Iizuka M, Matsumoto T, Saeki S, Yamaguchi H (1999) *In vitro* digestibility and fermentability of levan and its hypocholesterolemic effects in rats. J Nutr Biochem 10: 13-18.
25. Xu Q, Yajima T, Li W, Saito K, Ohshima Y, et al. (2006) Levan (beta-2, 6-fructan), a major fraction of fermented soybean mucilage, displays immunostimulating properties via Toll-like receptor 4 signalling: induction of interleukin-12 production and suppression of T-helper type 2 response and immunoglobulin E production. Clin Exp Allergy 36: 94-101.
26. Korakli M, Gänzle MG, Vogel RF (2002) Metabolism by *bifidobacteria* and lactic acid bacteria of polysaccharides from wheat and rye, and exopolysaccharides produced by *LactoBacillus sanfranciscensis*. J Appl Microbiol 92: 958-965.
27. Oliver AE, Leprince O, Wolkers WF, Hincha DK, Heyer AG, et al. (2001) Non-disaccharide-based mechanisms of protection during drying. Cryobiology 43: 151-167.
28. Faber EJ, Kamerling JP, Vliegenthart JF (2001) Structure of the extracellular polysaccharide produced by *LactoBacillus delbrueckii* subsp. *bulgaricus* 291. Carbohydr Res 331: 183-194.
29. Prajapati JB, Nair BM (2003) The history of fermented foods In: Fermented Functional Food. (Eds Edwards and R Farnworth) CRC Press 1–25.
30. Guven M, Yasar K, Karaca OB, Hayaloglu AA (2005) The effect of inulin as a fat replacer on the quality of set-type low-fat yogurt manufacture. Int J Dairy Technol 58: 180-184.
31. De Vuyst L, De Vin F, Vaningelgem F, Degeest B (2001) Recent *developments in* the biosynthesis and applications of heteropolysaccharides from lactic acid bacteria. Int Dairy J 11: 687-707.
32. Jolly L, Vincent SJF, Duboc P, Neeser JR (2002) Exploiting exopolysaccharides from lactic acid bacteria. Antonie van Leeuwenhoek 82: 367-374.
33. Wacher-Rodarte C, Galvan MV, Farres A, Gallardo F, Marshall VME, et al. (1993) Yogurt production from reconstituted skim milk powders using different polymer and non-polymer forming starter cultures. J Dairy Res 60: 247-254.
34. Folkenberg DM, Dejmek P, Skriver A, Skov Guldager H, Ipsen R (2006) Sensory and rheological screening of exopolysaccharide producing strains of bacterial yoghurt cultures. Int Dairy J 16: 111-118.
35. Behare P, Singh R, Singh RP (2009) Exopolysaccharide-producing mesophilic lactic cultures for preparation of fat-free Dahi - an Indian fermented milk. J Dairy Res 76: 90-97.
36. Kumar AS, Mody K, Jha B (2007) Bacterial exopolysaccharides--a perception. Journal of Basic Microbiology 47: 103-117.
37. van Kranenburg R, Boels IC, Kleerebezem M, de Vos WM (1999) Genetics and engineering of microbial exopolysaccharides for food: approaches for the production of existing and novel polysaccharides. Current Opinion in Biotechnology 10: 498-504.
38. Praveen K (2000) Physico-chemical and microstructural properties of dahi using EPS producing strains. M Sc Thesis, National Dairy Research Institute, Karnal, Haryana, India.
39. Dabour N, Kheadr E, Fliss I, LaPointe G (2005) Impact of ropy and capsular exopolysaccharide-producing strains of *Lactococcus Lactis* subsp. *cremoris* on reduced-fat Cheddar cheese production and whey composition. Int Dairy J 15: 459-471.
40. Florencia FS (2013) Rheology of spreadable goat cheese made with autochthonous lactic cultures differing in their ability to produce exopolysaccharides. Food Sci Technol 33: 233-238.
41. Yang Z, Li S, Zhang X, Zeng X, Li D, et al. (2010) Capsular and slime-polysaccharide production by *LactoBacillus rhamnosus* JAAS8 isolated from Chinese sauerkraut: Potential application in fermented milk products. J Biosci Bioeng 110: 53-57.
42. Hassan AN, Ipsen R, Janzen T, Qvist KB (2003) Microstructure and rheology of yogurt made with cultures differing only in their ability to produce exopolysaccharides. J Dairy Sci 86: 1632-1638.
43. Hassan AN, Frank JF, Farmer MA, Schmidt KA, Shalabi SI (1995) Observation of encapsulated lactic acid bacteria using confocal scanning laser microscopy. J Dairy Sci 78: 2624-2628.
44. Amatayakul T, Halmos AL, Sherkat F, Shah NP (2005) Physical characteristics of yoghurts made using exopolysaccharide-producing starter cultures and varying casein to whey protein ratios. Int Dairy J 16: 40-51.
45. Laws AP, Marshall VM (2001) The relevance of exopolysaccharides to the rheological properties in milk fermented with ropy strains of lactic acid bacteria. Int Dairy J 11: 709-722.
46. Obert G (1984) Manufacturing of technology of cream turo with extended shelf life & isolation of a special strain of lactic acid bacteria which improves product consistency (in Hungarian) Teijipar. 33: 47-48.
47. Tamime AY, Saarela M, Søndergaard AK, Mistry VV, Shah NP (2005) Production and maintenance of viability of probiotic micro-organisms in dairy products. In: Probiotic Dairy Products Blackwell: Oxford 39-72.
48. Duboc P, Mollet B (2001) Applications of exopolysaccharides in the dairy industry. Int Dairy J 11: 759-768.
49. Brandt M, Roth JK, Hammes WP (2003) Effect of an exopolysaccharide produced by *LactoBacillus sanfranciscensis* LTH1729 on dough and bread quality. In: L de Vuyst *Sourdough from fundamentals to applications* (p 80) Brussels: Vrije Universiteit Brussel (VUB) IMDO.
50. Korakli M, Rossmann A, Gänzle MG, Vogel RF (2001) Sucrose metabolism and exopolysaccharide production in wheat and rye sourdoughs by *LactoBacillus sanfranciscensis*. J Agric Food Chem 49: 5194-5200.
51. Galle S, Schwab C, Arendt E, Ganzle M (2010) Exopolysaccharide-forming *Weissella* strains as starter cultures for sorghum and wheat sourdoughs. J Agric Food Chem 58: 5834-5841.
52. Di Cagno R, De Angelis M, Limitone A, Minervini, P. Carnevali, A. Corsetti, M. Gänzle, R. Ciati, Gobbetti M (2006) Glucan and fructan production by sourdough *Weissella cibaria* and *LactoBacillus plantarum*. J Agric Food Chem 54: 9873-9881.
53. Nisha P, Ananthanarayan L, Singhal RS (2005) Effect of stabilizers on stabilization of idli (traditional south Indian food) batter during storage. Food Hydrocolloids 19: 179-186.
54. Thakur S, Prasad MS, Rastogi NK (1995) Effect of Xanthan on textural properties of idli (traditional south Indian food). Food Hydrocolloids 9: 141-145.
55. De Vuyst L, Degeest B (1999) Heterpolysaccharides from lactic acid bacteria. FEMS Microbiol Rev 23: 153-177.
56. Dudman WF (1977) In: Sutherland I (Ed) Surface carbohydrates of the prokaryotic cell. Academic press New York pp: 357-414.
57. Durlu-Ozkaya F, Aslimb B, Ozkaya MT (2007) Effect of exopolysaccharides (EPSs) produced by *LactoBacillus delbrueckii* subsp *bulgaricus* strains to bacteriophage and nisin sensitivity of the bacteria. LWT 40: 564–568.
58. Looijesteijn PJ, Trapet I, de Vries E, Abee T, Hugenholtz J (2001) Physiological function of exopolysaccharides produced by *Lactococcus Lactis*. Int J Food Microbiol 28: 71-80.
59. Lebeer S, Claes IJJ, Verhoeven TLA, Vanderleyden J, De Keersmaecker SCJ (2010) Exopolysaccharides of *LactoBacillus rhamnosus* GG form a protective shield against innate immune factors in the intestine. Microb Biotechnol 1-7.
60. Vanhooren P, Vandamme EJ (1998) Biosynthesis physiological role use and

fermentation process characteristics of bacterial exopolysaccharides. Recent research *developments in* fermentation and bioengineering 1: 253-299.

61. Cerning J (1990) Exocellular polysaccharides produced by lactic acid bacteria. FEMS Microbiol Rev 87: 113-130.

62. Lin TY, Chang Chien MF (2007) Exopolysaccharides production as affected by lactic acid bacteria and fermentation time. Food Chem 100: 1419-1423.

63. Rodríguez C, Medici M, Rodríguez AV, Mozzi F, Font de Valdez G (2008) Prevention of chronic gastritis by fermented milks made with exopolysaccharide-producing *Streptococcus thermophilus* strains. J Dairy Sci 92: 2423-2434.

64. Salazar N, Gueimonde M, Hernandez-Barranco AM, Ruas-Madiedo P, de los Reyes-Gavilan CG (2008) Exopolysaccharides produced by intestinal *Bifidobacterium* strains act as fermentable substrates for human intestinal bacteria. Applied and Environmental Microbiology 74: 4737-4745.

65. Nagaoka M, Hashimoto S, Watanabe T, Yokokura T, Moro Y (1994) Anti-ulcer effects of lactic acid bacteria and their cell wall polysaccharides. Biol Pharm Bull 17: 1012–1017.

66. Nakajima H, Hirota T, Toba T, Itoh T, Adachi S (1992) Structure of the extracellular polysaccharide from slime-forming *Lactococcus Lactis* subsp *cremoris* SBT 0495. Carbohydr Res 224: 245–253.

67. Soh HS, Kim CS, Lee SP (2003) A New *In vitro* Assay of Cholesterol Adsorption by Food and Microbial Polysaccharides. J Med Food 6: 225-230.

68. Tok E, Aslim B (2010) Cholesterol removal by some lactic acid bacteria that can be used as probiotic. Microbiol Immunol 54: 257 - 264.

69. Tsuda H, Hara K, Miyamoto T (2008) Binding of mutagens to exopolysaccharide produced by *LactoBacillus plantarum* mutant strain 301102S. J Dairy Sci 91: 2960-2966.

70. Whitfield C (1988) Bacterial extracellular polysaccharides. Can J Microbiol 34: 415-420.

71. Allison DG, Sutherland IW (1987) The role of exopolysaccharides in adhesion of fresh water bacteria. J Gen Microbiol 133: 1319-1327.

72. Hernández LJ, Arrieta C, Menéndez R, Vazquez A, Coego V, et al. (1995) Isolation and enzymic properties of levansucrase secreted by *Acetobacter diazotrophicus* SRT4 a bacterium associated with sugar cane. Biochemistry Journal 309: 113-118.

73. Russell RR (1990) Molecular genetics of glucan metabolism in oral streptococci. Archives of Oral Biology 35: 53S-58S.

74. De Palencia FP, Werning ML, Sierra-Filardi E, Duenas MT, Irastorza A, et al. (2009) Probiotic properties of the 2-substituted (1,3)-D-glucan-producing bacterium *Pediococcus parvulus* 2.6. Appl Environ Microbiol 4887-4891.

75. Klein MI, Duarte S, Xiao J, Mitra S, Foster TH (2009) Structural and molecular basis of the role of starch and sucrose in *Streptococcus mutans* biofilm development. Appl Environ Microbiol 75: 837–841.

76. Vishwanath K, Prajapati JB, Ljungh A (2012) Evaluation of adhesion of *LactoBacillus* strains to HT 29 cells by a flow cytometric assay. Int J App Ani Sci 1: 1-7

77. Oda M, Hasegawa H, Komatsu S, Kambe M, Tsuchiya F (1983) Anti-tumor polysaccharide from *LactoBacillus* spp. Agric Biol Chem 47: 1623-1625.

78. Forsén R, Heiska E, Herva E, Arvilommi H (1987) Immunobiological effects of *Streptococcus cremoris* from cultured milk 'viili'; application of human lymphocyte culture techniques. Int J Food Microbiol 5: 41-47.

79. Chabot S, Yu HL, De Leseleuc L, Cloutier D, van Calsteren MR, et al. (2001) Exopolysaccharides from *LactoBacillus rhamnosus* RW-9595M stimulate TNF, IL-6 and IL-12 in human and mouse cultured immunocompetent cells, and IFN-γ in mouse splenocytes. Lait 81: 683-697.

80. Kitazawa H, Harata T, Uemura J, Saito T, Kaneko T, Itoh T (1998) Phosphate group requirement for mitogenic activation of lymphocytes by an extracellular phosphopolysaccharide from *LactoBacillus delbrueckii spp bulgaricus*. Int J Food Microbiol 40: 169-175.

81. Gómez E, Melgar MM, Pérez Silva G, Portolés A, Gil I (1988) Exocellular products from *Bifidobacterium adolescentis* as immunomodifiers in the lymphoproliferative responses of mouse splenocytes. FEMS Microbiol Let 56: 47-52.

82. Kitazawa H, Yamaguchi T, Itoh T (1992) B-cell mitogenic activity of slime products produced from slime-forming, encapsulated *Lactococcus Lactis* ssp. *cremoris*. J Dairy Sci 75: 2946-2951.

83. Wang Y, Ganzle MG, Schwab C (2010) Exopolysaccharide synthesized by *LactoBacillus reuteri* decreases the ability of enterotoxigenic *Escherichia coli* to bind to porcine erythrocytes. Appl. Environ. Microbiol 76: 4863–4866.

84. Colliec-Jouault S, Chevolot L, Helley D, Ratiskol J, Bros A, et al. (2001) Characterization, chemical modifications and *In vitro* anticoagulant properties of an exopolysaccharide produced by *Alteromonas infernus*. Biochim Biophys Acta 1528: 141-151.

85. Vanhooren PT,Vandamme EJ (2000) Microbial production of clavan an L-fucose rich exopolysaccharide In: Food Biotechnology (Bielecki S Tramper J and Polak J eds) Elsevier Science BV Amsterdam The Netherlands, pp 109-114.

86. Liu CF, Tseng KC, Chiang SS, Lee BH, Hsu WH, et al. (2011) Immunomodulatory and antioxidant potential of *LactoBacillus* exopolysaccharides. J Sci Food Agric 91: 2284–2291.

87. Martin SA, Karnovsky ML, Krueger JM, Pappenheimer JR, Biemann K (1984) Peptidoglycans a promotors of slow-wave sleep I Structure of the sleep-promoting factor isolated from human urine. J Biol Chem 259: 12659-12662.

88. Patel AR, Lindström C, Patel A, Prajapati JB, Holst O (2012) Screening and isolation of exopolysaccharide producing lactic acid bacteria from vegetables and indigenous fermented foods of Gujarat India. International Journal of fermented foods 1: 87-101.

89. Ruas-Madiedo P, de los Reyes-Gavilán CG (2005) Invited Review: methods for the screening isolation and characterization of exopolysaccharides produced by lactic acid bacteria. J Dairy Sci 88: 843-856.

90. Wongsuphachat W, Kittikun AH, Maneerat S (2010) Optimization of exopolysaccharides production by *Weissella* confusa NH 02 isolated from Thai fermented sausages. Songklanakarin J Sci Tech 32: 27-35.

91. Rudén C (2004) Acrylamide and cancer risk-expert risk assessments and the public debate. Food Chem Toxicol 42: 335-349.

92. Cosa S, Mabinya LV, Olaniran OA, Okoh OO, Bernard K, et al. (2011) Bioflocculant Production by *VirgiBacillus* sp. Rob Isolated from the Bottom Sediment of Algoa Bay in the Eastern Cape, South Africaca. Molecules16: 2431-2442.

93. Mabinya VL, Cosa S, Nwodo UU, Okoh AI (2012) Studies on bioflocculant production by Arthrobacter sp Raats a freshwater bacteria isolated from Tyume River South Africa. Int J Mol Sci 13: 1054-1065.

94. Aparna SV, Shah N, Patel A (2013) Lactic acid bacteria as metal quenchers to improve Food safety and quality. Technical article published in souvenir of *Innovations in Dairy Industry* organised by SMC college of Dairy Science, AAU, Anand, Gujarat, India.

95. Qin G, Zhu L, Chen X, Wang PG, Zhang Y (2007) Structural characterization and ecological roles of a novel exopolysaccharide from the deep-sea psychrotolerant bacterium *Pseudoalteromonas* sp SM9913. Microbiology 153: 1566-1572.